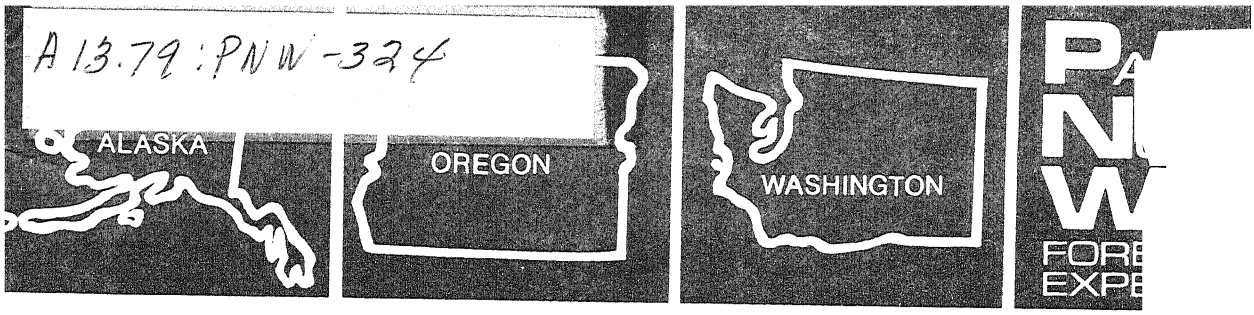

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Foliar Essential Oils and Deer Browsing Preference of Douglas-fir Genotypes

by

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ABSTRACT

Yield and composition of essential oils were compared in foliage of Douglas-fir. Five clones with different susceptibilities to deer browsing were used; foliage was collected during the dormant season. There were no qualitative differences among the oils of the different clones, but the oils differed quantitatively in all variables measured. Eight variables appeared useful in separating resistant from susceptible clones. Only one compound, however, an unidentified chemical, seemed capable of distinguishing between all three deer browsing preference classes.

KEYWORDS: Essential oils chemistry, browse preference, genotypes, Douglas-fir, *Pseudotsuga menziesii*, deer (black-tailed).

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INTRODUCTION

Feeding selection by black-tailed deer (*Odocoileus hemionus columbianus* Richardson) among Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) genotypes has been recently documented (Dimock et al. 1976). A universally accepted basis for such voluntary animal preferences, however, has yet to be established. Studies of factors affecting relative preference are necessary for a better understanding of plant-animal relationships and for devising methods of alleviating animal damage to forest trees.

Foliar essential oils and their terpenoid components have been prominent among the different chemical factors studied and have been postulated to influence feeding preference by deer (Radwan 1974). Important studies with Douglas-fir included determination of compositional changes in the oils during needle maturation (Maarse and Kepner 1970), evaluation of effects of the oils and their individual terpenes on deer rumen microbial activity *in vitro* (Oh et al. 1967, Oh et al. 1970, Radwan 1972), and determination of level and composition of volatile terpenes emitted from foliar essential oils (Radwan and Ellis 1975). There are no reports in the literature on relationships between content of whole essential oil or levels of its terpene components and selective deer browsing among foliage of different Douglas-fir trees.

In this study, therefore, I determined the yield and composition of the essential oils isolated from foliage of five different Douglas-fir clones previously ranked according to browsing preference by black-tailed deer. I compared clones by chemical component and attempted to identify the variables which were important in distinguishing between clones of the different deer browsing preference classes.

MATERIALS AND METHODS

PLANT MATERIAL

Test trees were from five different grafted clones (SD-8, SD-10, SD-13, SD-19, and SD-22) grown at the Olympic National Forest's Dennie Ahl Seed Orchard in western Washington. At time of sampling in 1974, the trees were 16 years old. Foliage from the clones had been ranked earlier into three preference classes according to browsing preference by captive deer--susceptible (SD-10 and SD-19), intermediate (SD-8), and resistant (SD-13 and SD-22) (Dimock et al. 1976).

Periodically, 100-g composite samples of foliage were collected from five trees of each clone in early morning during the dormant season. Each sample was obtained from current year's growth and consisted of 5-cm tips of secondary laterals cut from all sides of the trees at a height of about 1.5 m. Samples were individually sealed in precooled jars and brought to the laboratory in a portable cooler. There were 10 sample collections during the season.

CHEMICAL ANALYSES

In the laboratory, fresh foliage from each composite sample was chopped into small pieces. Subsamples were taken for moisture determination at 65°C, and isolation of essential oils.

Essential oils were obtained by blending tissue in minimum amounts of distilled water followed by steam-distillation for 4 hours in a Clevenger-type

apparatus (Clevenger 1928) and collecting the oils in n-heptane. The oil solutions were diluted to a common volume with heptane after adding n-tetradecane as internal standard; they were then transferred to airtight vials equipped with Teflon® 1/ septa and stored at -15°C until analyzed by gas-liquid chromatography.

Separations of the oil components were carried out with a gas chromatograph equipped with flame ionization detectors and two open tubular, stainless steel columns. Columns were 61 m by 0.05 cm (inner diameter) coated with a mixture of 95-percent Carbowax 20M plus 5-percent Igepal CO-880. Operating conditions were: injection port, 250°C; detector, 250°C; column, isothermal at 70°C for the first 3 min, programed to 150°C at 2°C/min, and held at 150°C for 8 min; and N_2, H_2, and air flows of 4, 25, and 250 ml/min, respectively. Resolved peaks were identified by comparing relative retention times of unknowns on two columns (Carbowax 20M and SF-96(50)) and their infrared spectra with those of unknown compounds and by peak enrichment. Compounds were quantified by measuring peak areas with electronic integrator. Average yields per gram of tissue for the individual clones were calculated based on the 10 samples collected and two injections per sample.

RESULTS AND DISCUSSION

Yields of the essential oils of the five clones and their terpene components in both the monoterpene hydrocarbon and oxygenated monoterpene regions are shown in table 1. The oils contained over 40 compounds each, but many were consistently present in small or trace amounts. Compounds identified were similar to those found earlier in foliar oils of Douglas-fir (Maarse and Kepner 1970); and as expected, the oils were predominantly (78-86 percent) composed of monoterpene hydrocarbons. Most abundant components present in the oils were α- and β-pinene, sabinene, 3-carene, δ-terpinene, and terpinolene in the monoterpene hydrocarbon region, and terpinen-4-ol and α-terpineol in the oxygenated monoterpene region.

There were no qualitative differences among the oils of the different clones. In all collections, the same terpene components were detected in oils of the five clones.

Quantitative differences between clones were apparent in all terpenes comprising the oils, also in the sums of terpenes in the monoterpene hydrocarbon region, the sums of compounds in the oxygenated monoterpene region, and total yields of all terpenes. Levels of these variables, therefore, are characteristic of the different clones.

Comparisons of the different foliar oils indicated eight variables which appeared useful in distinguishing between clones of the different preference classes. These indicators of preference included the unknown compound represented by peak 30, β-phellandrene, linalool, citronellyl acetate, α-terpineol, geranyl acetate, the sum of terpenes in the monoterpene hydrocarbon region, and the total terpene yield. Level of the unknown compound (peak 30) was highest in the susceptible clones, intermediate in the oil of clone SD-8, and lowest in foliage of the two resistant genotypes. Remaining indicators, on the other hand, were higher in the resistant than in the susceptible clones, but not strictly intermediate in the foliage of the clone with the intermediate susceptibility to browsing

1/ Trade names mentioned do not constitute endorsement by the U.S. Department of Agriculture over similar products.

Table 1--*Composition and yield of foliar essential oils of different Douglas-fir clones*[1/]

Peak number	Component	Peak area (x 10^6)				
		Clone SD-10	Clone SD-19	Clone SD-8	Clone SD-13	Clone SD-22
	MONOTERPENE HYDROCARBON REGION					
2	α-pinene	17.14	25.38	20.88	27.16	31.60
3	Camphene	1.66	3.78	1.16	1.04	2.79
4	β-pinene	34.59	55.94	41.36	57.17	90.90
5	Sabinene	14.02	3.78	11.60	15.48	7.94
6	Δ-3-carene	13.43	3.56	9.12	8.80	8.94
7	Myrcene + α-phellandrene	4.77	2.96	3.38	4.28	5.23
9	α-terpinolene	7.51	1.40	5.34	5.72	2.87
10	Limonene	2.19	4.29	3.48	3.69	8.03
11	β-phellandrene	2.86	1.85	2.40	3.08	3.46
14	δ-terpinene + unknown	13.53	4.17	11.96	11.68	6.42
16	Terpinolene + p-cymeme	29.28	7.10	22.80	25.82	13.14
	Total	140.98	114.21	133.48	163.92	181.32
	OXYGENATED MONOTERPENE REGION					
20	Cintronellal	0.08	0.09	0.09	0.06	0.48
21	Linalool	.33	.32	.16	1.31	.43
22	Unknown	1.11	.91	.72	.79	.92
25	Unknown	.75	2.28	.59	.50	1.01
27	Terpinen-4-ol	20.40	4.25	17.53	17.72	7.50
30	Unknown	1.72	1.15	.94	.84	.38
31	Citronellyl acetate	1.58	1.05	2.16	1.60	3.14
32	Unknown	1.59	1.40	.57	1.34	7.38
33	α-terpineol	4.19	3.70	5.45	5.32	5.38
35	Unknown	1.03	.89	.36	.71	1.45
37	Citronellol	.25	.28	.20	.24	.88
38	Geranyl acetate	.98	.40	.92	2.84	2.70
--	Other unknowns	5.09	2.38	2.51	4.04	3.61
	Total	39.10	19.10	32.20	37.31	35.26
Total, both regions		180.08	133.31	165.68	201.23	216.58

[1/] Components measured in arbitrary units determined by electronic integrator and calculated per gram of foliage tissue. Values are means of 10 composite samples from five trees each. Susceptibility to deer browsing: SD-10 and SD-19 susceptible, SD-8 intermediate, SD-13 and SD-22 resistant.

(SD-8). The unknown compound, therefore, appeared to be the most sensitive indicator of deer browsing preference.

CONCLUSIONS

The five clones investigated in this study varied in yield and composition of the essential oils of their foliage. Clearly such characteristics of the foliar oils are among the chemical traits which show genetic variation in Douglas-fir, and their determination can be a valuable tool in identifying different genotypes.

Eight variables appeared useful in separating the resistant from the susceptible clones. Only one compound, however, an unidentified chemical, seemed capable of distinguishing between all three preference classes.

Expansion upon the findings of this study is needed. Most probably, measuring deer browsing preference by some continuous variable and increasing the number of clones studied would be desirable. This would permit use of sensitive statistical methods such as discriminant analysis (Rao 1952) to isolate terpenes and terpene ratios with the greatest discriminating ability among foliage of varying susceptibilities to deer browsing. Additionally, studies should be continued to evaluate other chemical compounds, such as chlorogenic acid, which have been shown to be positively associated with palatibility of Douglas-fir (Radwan 1975, Tucker et al. 1976). Such biochemical research, coupled with use of advanced statistical methods, could ultimately lead to development of chemical indicators of resistance to browsing and to practical programs to alleviate deer browsing on Douglas-fir.

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